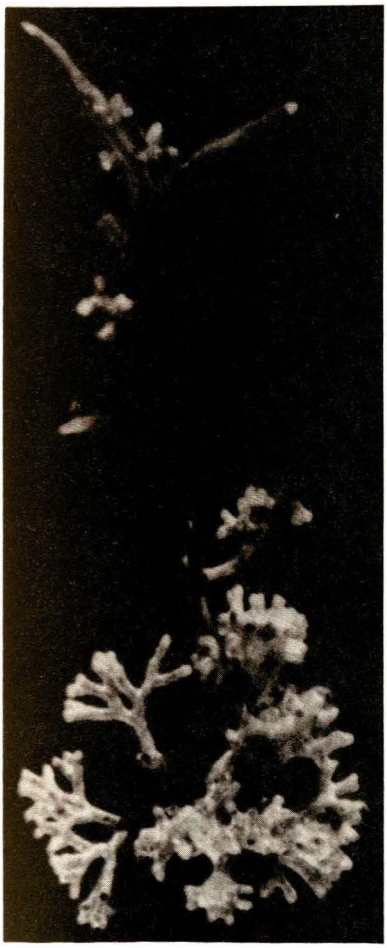
Relation of soil nutrients
and light to prevalence of

MYCORRHIZAE

on pine seedlings



by

Edward Hacskaylo
Albert G. Snow, Jr.

STATION PAPER NO. 125 • NORTHEASTERN FOREST EXPERIMENT STATION • 1959
FOREST SERVICE • U.S. DEPARTMENT OF AGRICULTURE • UPPER DARBY, PA.
RALPH W. MARQUIS, DIRECTOR

Relation of soil nutrients
and light to prevalence of

MYCORRHIZAE

on pine seedlings

---

by Edward Hacskaylo
Albert G. Snow, Jr.

About the authors . . .

Edward Hacskaylo took his Bachelor's, Master's, and Ph.D. degrees in botany at George Washington University, the last in 1954. His graduate work included a year at the Institute of Physiological Botany, University of Uppsala, Sweden, studying the physiology of mycorrhizae on a fellowship from the American Scandinavian Foundation. Dr. Hacskaylo has been with the U.S. Department of Agriculture since 1954, when he joined the Ornamental Plants Section of the Agricultural Research Service. In 1958, he transferred to the Forest Service and the Forest Tree Physiology Laboratory in Beltsville, Maryland. His primary investigations have dealt with the physiology of mycorrhizal relations.

Albert G. Snow, Jr. was graduated from Washington State University in 1933 with a Bachelor's degree in forestry and from Yale University 2 years later with a Master's degree in tree physiology and plant pathology. In 1935 he joined the U.S. Forest Service, Northeastern Forest Experiment Station in New Haven, doing research on spruce management and forest genetics. Then for 10 years he served with the Southeastern Forest Experiment Station in Lake City, Florida, working on naval stores. He returned to the Northeastern Station in 1952 for assignment at the Station's research center at Laurel, Md. He is now center leader at Laurel.

Introduction

ODD RELATIONSHIPS abound among the wonders of Nature. Just as men keep cows, some ants herd aphids. The bee pays Nature for its food by pollinating the flower. One symbiotic relationship that concerns foresters is the coupling of tree roots with certain fungi to form compound structures, part tree root and part fungus mycelium, which we call mycorrhizae.

Mycorrhizae become, in effect, part of the tree; and to some degree they influence tree growth. And they in turn seem to be influenced in complex ways that we do not fully understand by the nutrients and light that trees demand.

Research has produced some information about this. Hatch (1937), Mitchell et al (1937), Bjorkman (1942, 1949), and Doak (1955) found that high levels of available nutrients, especially nitrogen and phosphorus, reduced the abun-

dance of mycorrhizae. Conversely, when the nutrient level was moderate or relatively low, mycorrhizal fungi invaved the short roots.

Bjorkman (1942) also published evidence that fewer mycorrhizae form on pines subjected to low light intensities than on those grown at high intensities. He found that, at light intensities less than 23 percent of full daylight, the development of mycorrhizae on Scotch pine varied greatly in different soils depending upon available nutrients. However, at 6 percent of full sunlight mycorrhizae never formed. Bjorkman concluded that an excess of soluble carbohydrates in roots encourages invasion by the fungal hyphae. Conversely, it may be assumed that little or no invasion by mycorrhizal fungi would occur when soluble carbohydrates in the roots are low. This situation may prevail when soil nutrients are abundant and growth vigorous; then most of the newly synthesized carbohydrates would be utilized in tissue formation, with little accumulation in the roots.

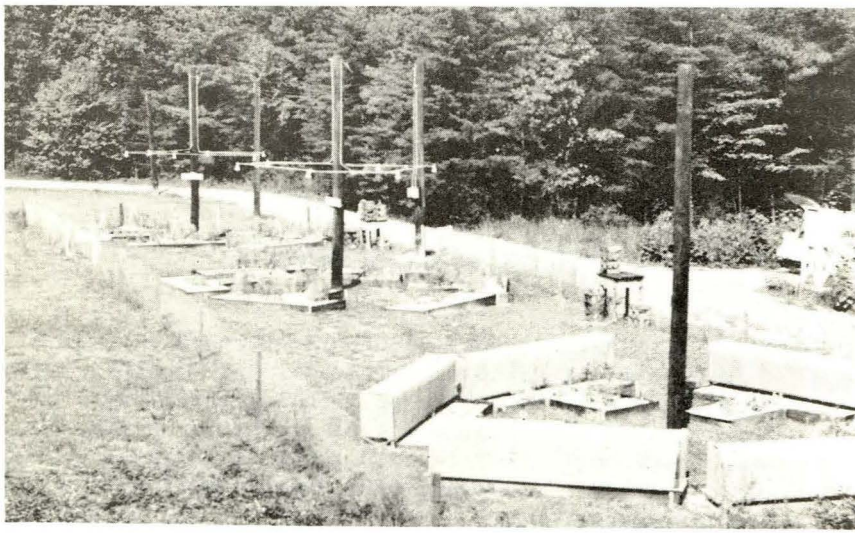


Figure 1.--*Experimental field set-up for study of light and nutrition. The group of replicate beds in the foreground received the natural season photoperiod, with half the bed shaded to give a light intensity one-tenth of full sunlight.*

The two groups in the background received extra light at night from 150-watt incandescent floodlights suspended 7 feet above the center of each bed.

From a study of the influence of different levels of daylight radiation on the growth and nature of mycorrhizal infection of beech seedlings, Harley and Waid (1955) obtained results very similar to those that Bjorkman found.

But no information is available in the literature about the combined effect of nutrition and the intensity and duration of light on the frequency of mycorrhizae on Virginia, loblolly, and white pines.

This paper is an attempt to contribute some information to help fill the gap. However, we must point out that the experiments on which this paper is based were designed primarily to study how nutrition influences the photoperiodic responses of these three species (fig. 1). For this reason our data on mycorrhizal relationships are not as complete as we would like them to be.

Materials & Methods

Seedlings of Virginia pine (*Pinus virginiana* Mill.), loblolly pine (*P. taeda* L.), and eastern white pine (*P. strobus* L.) were examined in November 1956 after they had grown outdoors for approximately 5 months in clay pots set in sand plots at the Beltsville Experimental Forest. There were five seedlings per 8-inch pot. Some plots had been exposed to natural daylengths without shade, others to natural daylengths with shade from burlap, and still others to natural daylengths plus light from incadescent bulbs during parts of the dark period. Four levels of soil nutrition were provided, and there were three replications under each condition of light.

Nutrient Levels

Sassafras sandy loam, a soil common to northern portions of the Coastal Plain, was used in these experiments (treatment designated as N_2). Judged from crop standards, the general fertility level of this soil is low. Soil analyses by the Maryland State Soils Testing Laboratory indicated that at the start of the experiment available amounts of three common elements were: magnesium (Mg), 11 ppm (parts per million); phosporous (P), 11 ppm; potassium (K), 23 ppm.

In one treatment series (designated N_1), one part of this soil was mixed with nine parts of a quartz sand. The particle size was similar to the sand size of the Sassafras sandy loam.

In two other treatments (N_3 and N_4), 500 milliliters of a nutrient solution were added once a week to the soil in each pot. The concentrations of the cations and anions used in treatment N_3 were: calcium (Ca), 80 ppm; magnesium (Mg), 36 ppm; potassium (K), 195 ppm; nitrate (NO_3), 310 ppm; sulphate (SO_4), 96 ppm; mono-hydrogen phosphate (HPO_4), 144 ppm. In treatment N_4, the solution was three times the concentration used in N_3.

At the end of the study, analyses of the soil receiving added nutrients were made on the same basis as the original sample. The concentrations of elements were:

Nutrient level	*Magnesium (ppm)*	*Phosphorus (ppm)*	*Potassium (ppm)*
N_3	68	26	78
N_4	73	28	117

These results, contrasted to those for analyses of the original soil, show that the general nutrient levels of both series were increased considerably during the period of the experiment.

Light Exposures

All seedlings received sunlight for the natural photoperiod during the day. One group, including each species at each nutrient level, received full sunligh; another was shaded so that the light intensity was about one-tenth that of full sunlight. Two other groups were given extra hours of light, one by extending the natural day-length, the other by interruption of the dark period, as described below.

The supplemental light was supplied by incadescent-filament lamps. For the extended-day treatment (ED), extra light was supplied in equal amounts before sunrise and after sunset to provide the equivalent of a 20-hour day. For the interrupted dark treatment (ID), light was supplied for 2 hours in the middle of the night. Subgroups within each treatment received the extra light at intensities of either 45 foot-candles or 15 foot-candles (fig. 2).

Figure 2.--*Seedlings in a bed that received supplemental light. The group of pots in the foreground are directly under an overhead light; they received light of 45 foot-candle intensity. The semicircle of pots in the background received extra light from the same source, but at an intensity of 15 foot-candles.*

Mycorrhizae Examination

When the seedlings were 5 months old they were removed from the pots, and all soil was carefully washed from the roots. The general characteristics of the roots were noted, and the percentages of the short roots converted to mycorrhizae on the root systems were estimated for each treatment. Samples of side roots from average seedlings in each group were put in Navashin fixative for later examination.

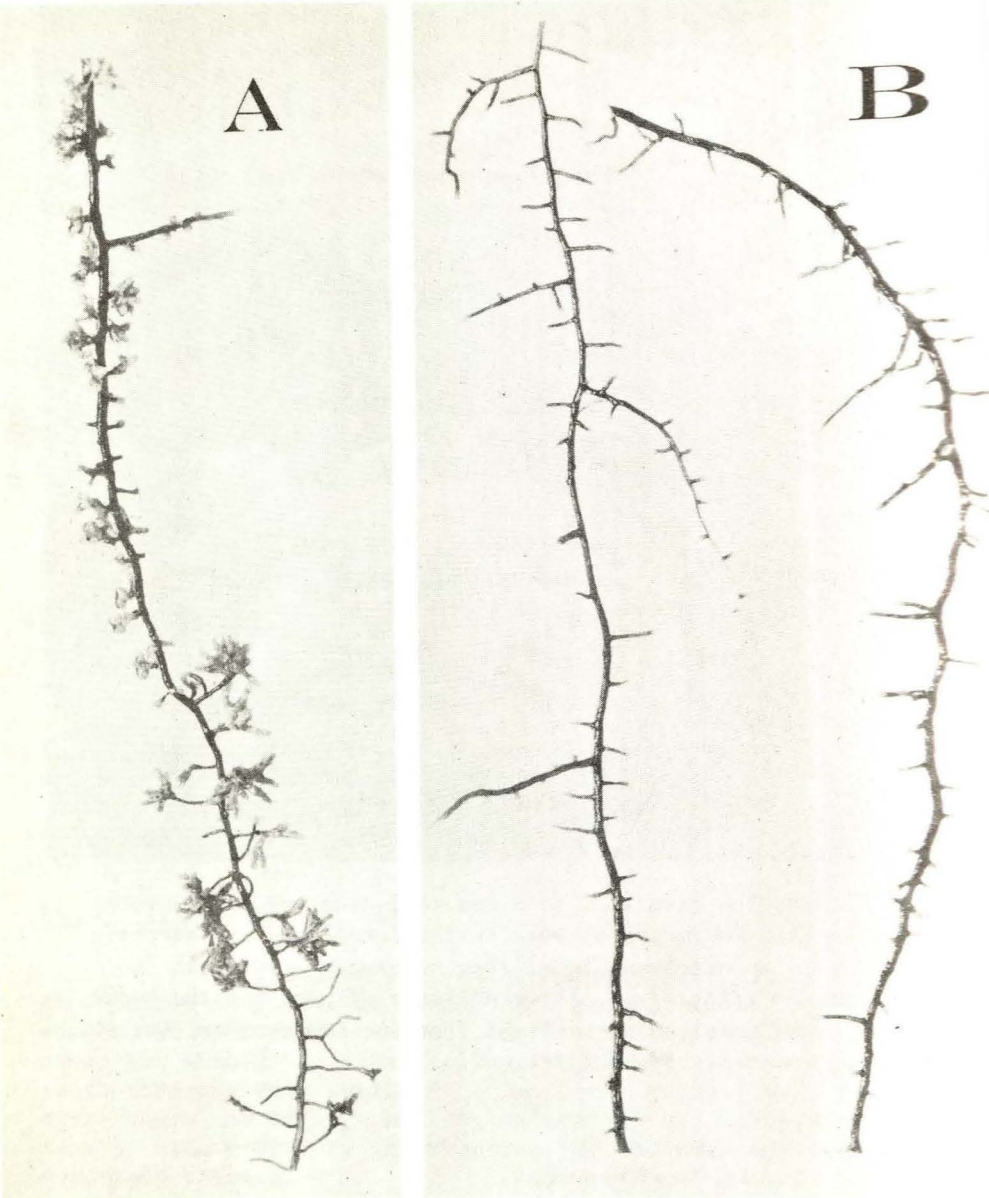


Figure 3.--*Long roots of Virginia pine grown in Sassafras sandy loam in full sunlight and natural daylengths.*

A, *No nutrients added (N_2 level); note the heavy development of mycorrhizae.* B, *Nutrients added (N_4 level); note the absence of mycorrhizae.*

Results

Effects of Nutrient Level on Prevalence of Mycorrhizae

The root systems of seedlings grown at the N_1 nutrient level were consistently smaller than those grown at the higher levels. Extensive root systems developed at the N_2, N_3, and N_4 levels, with a slight tendency toward larger roots under N_3 and N_4.

The heaviest formation of mycorrhizae occurred at the N_2 level (fig. 3). Mycorrhizae formation was generally less at the N_1 level under all light treatments; however, under natural light, mycorrhizae formed in fair abundance at this level (tables 1 and 2). Additions of nutrient solution reduced the prevalence of mycorrhizae. At the N_3 level, less than 10 percent of the short roots were mycorrhizal (table 2) except in two instances, one each of white pine and Virginia pine. At the N_4 level, mycorrhizae were seldom evident (fig. 3).

Table 1.--Relative abundance of mycorrhizae on the roots of pine seedlings grown in full sunlight or in shade with variations in nutrient levels

Light condition and nutrient level		Prevalence of short roots with mycorrhizae		
		Virginia pine	Loblolly pine	Eastern white pine
		Percent	Percent	Percent
Full sunlight	N_1	10-20	40-50	80-90
	N_2	30-40	70-80	80-90
	N_3	1-10	1-10	0
	N_4	0	1-10	0
Shade[1]	N_1	0	0	0
	N_2	0	1-10	0
	N_3	1-10	1-10	0
	N_4	0	0	0

[1]Shaded seedlings received about 10 percent of full sunlight. The photoperiod was natural for the season.

Table 2.--Relative abundance (in percent[1]) of mycorrhizae on the roots of pine seedlings grown at different nutrient levels with variations in supplemental light treatments

Light condition and nutrient level		Prevalence of short roots with mycorrhizae					
		Virginia pine at--		Loblolly pine at--		Eastern white pine at--	
		45 fc	15 fc	45 fc	15 fc	45 fc	15 fc
Interrupted dark[2]	N_1	5	5	45	5	25	5
	N_2	65	15	65	65	85	5
	N_3	5	55	5	5	25	0
	N_4	5	0	0	0	0	0
Extended day[3]	N_1	5	0	25	15	0	0
	N_2	45	15	75	35	5	35
	N_3	5	0	5	5	0	0
	N_4	0	0	0	5	0	0

[1]Prevalence indicated at the midpoint of a 10-percent class.

[2]Seedlings received 2 hours of extra light in the middle of the night.

[3]Seedlings received extra light in the morning and evening to make equivalent of a 20-hour day.

The mycorrhizae varied in form from simple to dichotomously branched to carolloid. In color, the fungus mantles were white, gray, or black; the last probably were *Cenococcum graniforme*. Black-mantled mycorrhizae were observed on plants grown at the N_3 level.

Effects of Light Differences on Formation of Mycorrhizae

Mycorrhizae developed best on plants in full sunlight under natural daylengths. This was best demonstrated by the N_1 and N_2 nutrient levels; as noted above, few mycorrhizae formed on N_3 and N_4 plants under any light regime. On shaded seedlings the formation of mycorrhizae was much less than on seedlings with full sunlight (table 1). The few that did form were found only at the middle levels of nutrition (N_2 and N_3).

Increasing the photoperiod, either by extending the daylength or interrupting the dark period, changed the abundance of mycorrhizae. This change varied with species and was dependent on the intensity of extra light received (tables 1 and 2). Extra light at the 45 foot-candle level did not greatly change the relative abundance of mycorrhizae in Virginia and loblolly pines, compared to the amounts on seedlings of these species receiving natural photoperiods in full sunlight. Eastern white pine was an exception: my-

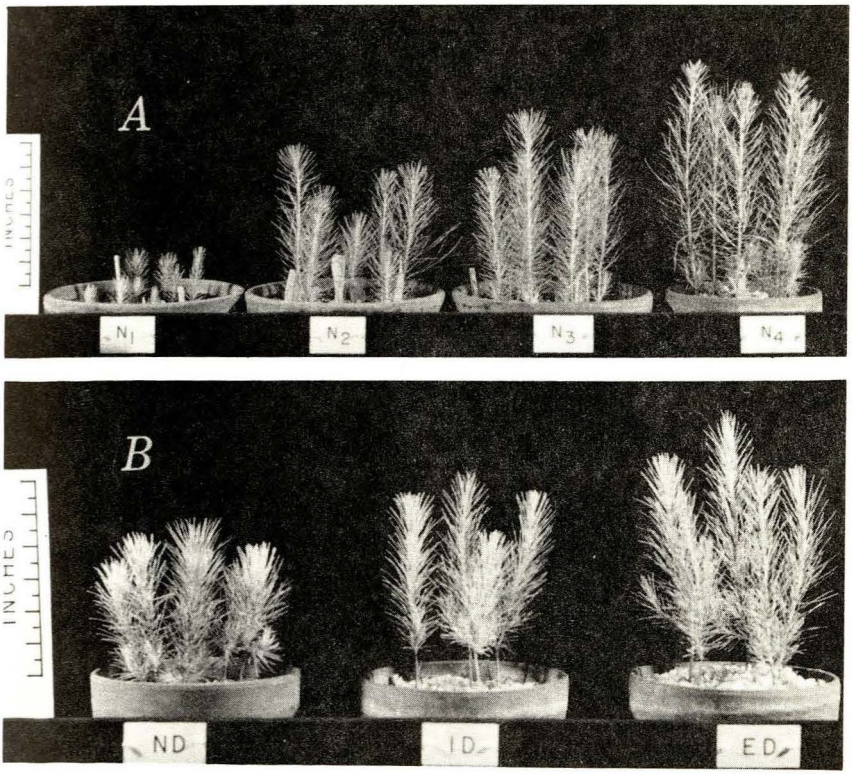


Figure 4.--*Height-growth response of seedlings in the field experiment.*

A, *Loblolly pine grown with different nutrient treatments (N_1, N_2, N_3, N_4) on a 20-hour day (ED) at a light intensity of 15 foot-candles.*

B, *Virginia pine grown under different light treatments with full sun (ND), and with extra light at an intensity of 45 foot-candles (ID and ED), with all at a very high nutrient level (N_4).*

corrhizae on the short roots of seedlings under a 20-hour photoperiod were generally less abundant than under the natural photoperiod, or with light interrupting the dark period. In most instances there was a sharp reduction in mycorrhizae when the extra light was at an intensity of 15 foot-candles compared to a light intensity of 45 foot-candles.

Prevalence of Mycorrhizae on Different Species

Virginia pine formed fewer mycorrhizae than the other two species under full sunlight on a natural daylength. When the daylength was extended 4 hours, making the equivalent of a 20-hour day, mycorrhizal formation was greater for loblolly pine than for the other two species. Under long days mycorrhizae were least abundant on eastern white pine.

Nevertheless, these observations on species differences should be considered as indicating trends only. The data were too variable to offer conclusive proof of specific species differences.

Discussion & Conclussions

Our observations of the roots of the seedlings in this experiment are in general agreement with those of other investigators. They have reported that the formation of ectotrophic mycorrhizae is strongly influenced by the concentration of nutrients available in the substrata and by the intensity of the light under which the plants are grown.

The very low levels of substrata nutrients (both organic and inorganic) in the N_1 group probably limited the growth of the fungi as well as that of the hosts. Even though a few short roots were formed, the fungi were not vigorous enough to encompass and invade all short roots.

In the N_2 group (unsupplemented Sassafras sandy loam), the nutrient levels were sufficient to permit heavy mycelial growth, but limited the growth of the pines (fig. 4). Increasing the daylength at this nutrient level caused no change in the abundance of mycorrhizae.

Low light intensities were a limiting factor in the formation of mycorrhizae on both natural and long days. As Bjorkman (1942) showed, the carbohydrate content of roots is reduced under these conditions. Consequently, one would assume that a low level of carbohydrates at low light intensities reduced the amount of the growth-promoting metabolite

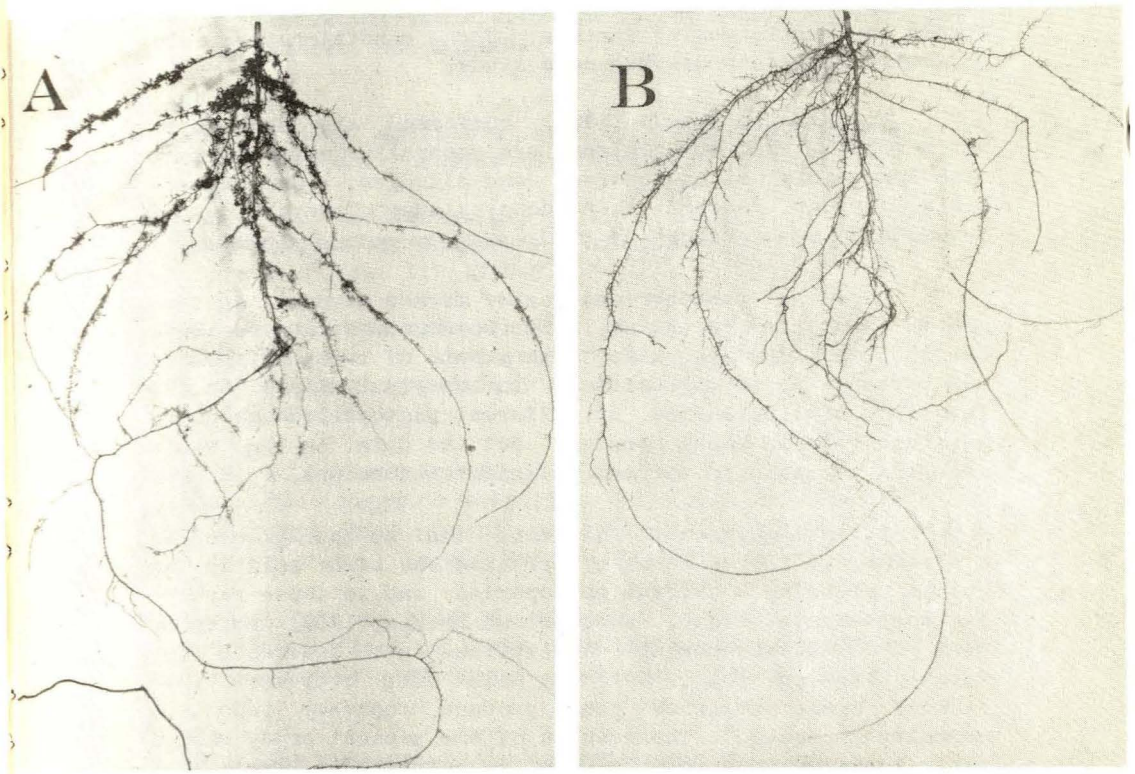


Figure 5.--*Relative abundance of mycorrhizae on roots of loblolly pine seedlings grown under a 20-hour daylength. A, Grown in Sassafras sandy loam with no nutrients added (N_2). B, Grown in the same soil with nutrients added at a high level (N_4).*

secreted by the roots, as indicated by Melin (1954). This condition does not promote hyphal development on and within the short roots. Increasing the level of nutrients compensated only in part for the retarding effects of low light intensities on mycorrhizal formation.

Possibly the poor development of mycorrhizae at the higher nutrient levels (N_3 and N_4) can be attributed to the same basic condition, near-depletion of soluble carbohydrates in the roots and reduced secretion of growth substances. In this case, the high nutrient levels stimulated root growth, which would have entailed rapid assimilation of carbohydrates, and thus could have precluded carbohydrate accumulation.

The conditions that permit and promote formation of mycorrhizae no doubt are controlled by a complex of both internal and environmental factors. These conditions are only partially known and deserve more study.

Fowells and Krauss (1959) confirmed the findings of Hatch (1937) that mycorrhizae are generally more abundant with low levels of phosphorous and nitrogen. Our experiments further support these conclusions: prevalence of mycorrhizae was greatest in soils with no nutrient added.

Different photoperiods cause marked changes in tree growth (Downs and Borthwick, 1956; Downs and Piringer, 1958). Long photoperiods also affect the growth of roots (fig. 5), and perhaps their metabolism. In our observations on the formation of mycorrhizae at different photoperiods we noted possible trends among species, but the data on the whole were too variable to warrant definite conclusions.

It is significant, however, that at the N_2 level of nutrition the incidence of mycorrhizae was high both in the groups receiving a natural photoperiod and in those receiving supplemental light. Fowells and Krauss (1959) indicated that mycorrhizal-bearing root systems are desirable for forest planting stock. But, they point out, "over-fertilization of forest nurseries could produce topheavy trees with undesirable roots." The results of the present study showed that long photoperiods at the N_2 nutritional level will greatly increase height growth and stem diameters of loblolly and Virginia pine seedlings (fig. 4). The root systems are correspondingly larger too; and they have abundant mycorrhizae.

The complex of the effects of nutrition and light on all aspects of the growth of tree seedlings is still little understood. Both of these factors, separately and in combinations, affect the mycorrhizal associations of roots of seedlings. These associations in turn influence tree growth to some degree. We hope others will assist in unraveling these complicated relationships so that better methods of handling tree species in their seedling stages can be formulated.

Literature Cited

Bjorkman, E.
1942. Über die Bedingungen der Mykorrhizabildung bei Kiefer und Fichte. Symb. Bot. Upsal 6 (2): 1-191.

1949. The ecological significance of the ectotrophic mycorrhizal association in forest trees. Svensk Bot. Tidskr. 43: 223-262.

Doak, K. D.
1955. Mineral nutrition and mycorrhizal association of bur oak. Lloydia 18: 101-108.

Downs, R. J., and Borthwick, H. A.
1956. Effects of photoperiod on growth of trees. Bot. Gaz. 117: 310-326.

---------- and Piringer, Jr., A. A.
1958. Effects of photoperiod and kind of supplemental light on vegetative growth of pines. Forest Sci. 4: 185-195.

Fowells, H. A., and Krauss, R. W.
1959. The inorganic nutrition of loblolly and Virginia pine with special reference to nitrogen and phosphorous. Forest Sci. 5: 95-112.

Harley, J. L,, and Waid, J. S.
1955. The effect of light upon the roots of beech and its surface population. Plant and Soil 7: 96-112.

Hatch, A. B.
1937. The physical basis of mycotrophy in Pinus. Black Rock Forest Bul. 6. 168 pp., illus.

Melin, E.
1954. Growth factor requirements of mycorrhizal fungi of forest trees. Svensk Bot. Tidskr. 48: 86-94.

Mitchell, H. L., Finn, R. F., and Rosendahl, R. O.
1937. The relation between mycorrhizae and the growth and nutrient absorption of coniferous seedlings in nursery beds. Black Rock Forest Papers 1: 58-73.